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***SPECIFIC WESTERN BLOT BANDS ARE ASSOCIATED  
WITH INITIAL CD4+ LYMPHOCYTE COUNTS IN  
HUMAN IMMUNODEFICIENCY VIRUS SEROCONVERTERS***

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## Specific Western Blot Bands Are Associated With Initial CD4+ Lymphocyte Counts in Human Immunodeficiency Virus Seroconverters

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## Summary

*Background.* The Western blot is the most widely used confirmatory test for diagnosis of human immunodeficiency virus (HIV) seropositivity. Specific bands in the Western blot indicate antibody responses to various portions of HIV or its precursors, and each is assigned a score from 0 to 3+. While the precise role of humoral antibody responses has not been fully established, specific antibody responses might influence the course of HIV infection.

*Methods.* This study investigates the association between antibody reactivity to nine principal Western blot bands and initial CD4+ counts among 877 Navy and Marine Corps personnel during 1988 to 1991. Multiple regression was used to evaluate the strength and significance of the associations and to adjust for age and estimated duration of infection.

*Results.* Strong antibody responses to the p24 core ( $p < 0.05$ ), p53 reverse transcriptase ( $p < 0.005$ ), and p55 core precursor ( $p < 0.0001$ ) antigens were associated with higher initial CD4+ counts, with 33 to 48 additional cells per  $\text{mm}^3$  associated with each unit increase in the Western blot score, according to a multiple regression analysis which controlled for age and duration of infection (maximum 24 months). By contrast, antibodies to the gp41 transmembrane antigen ( $p < 0.0001$ ) were associated with lower initial CD4+ counts. Each unit increase in the gp41 band was associated with 76 fewer CD4+ cells per  $\text{mm}^3$ . A negative association was also observed for the gp160 envelope precursor antigen, with each unit increase in reactivity associated with 51 fewer CD4+ cells, although this association was not statistically significant ( $p = 0.09$ ). Antibodies to the p17 core precursor, the p31 endonuclease, the p64 reverse transcriptase, and the gp120 envelope, were not statistically significantly associated with the CD4+ count in the multiple regression analyses.

*Conclusions.* The positive associations found for p24, p53, and p55 antibodies and CD4+ counts, and the negative association with gp41 antibodies, may have implications in development of a therapeutic vaccine for HIV seropositive patients.

## Introduction

The Western blot includes nine principal bands which represent the presence of antibodies to various viral components [1,2] and is the main confirmatory test for the diagnosis of HIV infection [3-6]. It has been reported that almost all individuals exposed to HIV from transfused blood will develop antibodies within three months [7], but it is unclear whether the nature of the antibody response influences the rate of loss of CD4+ lymphocytes or progression to AIDS, and the prognostic implications of specific antibodies to HIV remain uncertain [8-10]. Persons infected with HIV have antibodies that mediate antibody-dependent cellular cytotoxicity against HIV-infected target cells, and declines in serum antibodies to p24 and p17 have been associated with loss of antibody-dependent cytotoxicity [8]. Poor prognosis is associated with loss of reactivity in the p24 [8] and p17 [11] bands, which represent antibodies to products of the HIV core (*gag*) gene [12]. Although patterns of reactivity in the gp41, gp120, and gp160 bands during HIV infection have been previously described [6,9,10], specific associations of these bands with initial CD4+ lymphocyte counts following HIV seroconversion have not previously been reported.

The CD4+ lymphocyte count is a principal marker of HIV disease status and prognosis in apparently healthy HIV seroconverters, with a low count indicating a poor prognosis [3,6,13]. A decline in CD4+ lymphocyte counts has been reported to occur at or soon after HIV seroconversion [14]. There is a broad range of initial CD4+ counts near the time of seroconversion [15,16], and a uniform decline in these counts does not appear to be universal [14]. Relative freedom from life-threatening HIV-associated disease is usually present while the CD4+ lymphocyte count remains above 200 cells/mm<sup>3</sup> [13]. This study investigates the association of Western blot bands and initial CD4+ lymphocyte counts in active-duty U.S. Navy and Marine Corps personnel with documented HIV seroconversion during 1988 to 91.

## Methods

**Population.** The Navy HIV Central Registry maintained at the Naval Health Research Center in San Diego contains results from the Navy HIV Screening Program and from routine follow-up clinical evaluations of HIV seropositive personnel. Seropositive personnel were identified from the service-wide HIV testing program for all active-duty Navy and Marine Corps personnel, testing of individuals in preparation for deployment overseas, testing of health care providers, routine physical examinations, or clinical evaluations.

Computerized records of all personnel who received negative HIV enzyme-linked immunoassay (EIA) tests from January 1, 1988 through December 12, 1991 were used to determine the population at risk for seroconversion. Testing procedures have been described in detail elsewhere [17,18]. Seroconverters were required to have a positive Western blot test meeting Centers for Disease Control criteria for HIV positivity [19], with at least two of three bands present at p24, gp41, and gp120/160. A minimum of 30 days was required between the last negative EIA test and the first positive Western blot. The

midpoint of the time interval between an individual's most recent negative EIA test and first positive Western blot was used as the estimated seroconversion date, as in previous studies [15,16,20,21]. No more than two years was allowed to elapse between the estimated date of seroconversion and the initial CD4+ count.

*Western blot testing.* The possible values for density of the Western blot bands (a marker of the intensity of the antibody response) were: 0, nonreactive; 1+, weakly reactive; 2+, moderately reactive; and 3+, strongly reactive. Scoring of Western blot bands was performed during January 1, 1988 to June 30, 1989 by Biotech Laboratories (Rockville MD), and during July 1, 1989 to December 12, 1991 by North American Biologicals, Inc. (Miami FL) using an FDA-licensed test developed by the National Institutes of Health.

*CD4+ lymphocyte counts.* CD4+ counts on initial clinical evaluation were obtained from the Navy HIV Central Registry [17,18] which contains the results of all Navy clinical evaluations. Evaluations were performed at Naval Medical Centers in Bethesda, MD; San Diego, CA; Portsmouth, VA; and Oakland, CA. CD4+ counts were performed using laser-based flow cytometry: a Coulter Profile 1 (Coulter Corporation, Hialeah, FL) was used in Bethesda; a Coulter Epics Model C in Portsmouth; and a Becton-Dickinson (Immunocytometry Systems Division, San Jose CA) FACScan in San Diego and Oakland. Hematology was performed in all laboratories using a Coulter counter (Coulter Corporation, Hialeah FL). Analyses were performed within each hospital, except Oakland, where they were performed at the Clinical Laboratories of the University of California, San Francisco, Medical Center, and at Naval Medical Center, Bethesda, during 1988 through mid-1990, when they were performed at Smith-Kline Beecham Clinical Laboratories in Rockville MD.

The flow cytometry and quality assurance procedures used in this study have been described previously [15,16,22]. All laboratories (except the University of California, San Francisco, Medical Center) were participants in an ongoing comprehensive quality assurance program which provided monthly aliquots of human blood, standardized quality-control reagents, and monthly proficiency testing [22]. The laboratories were required to provide monthly CD4+ counts on these specimens, which were used to determine coefficients of variation. Hematology procedures for lymphocyte counts also were standardized and reported monthly for multiple identical aliquots of human blood. Coefficients of variation among laboratories for CD4+ counts were available for 1988-1991: they were 19 percent in 1988, 10 percent in 1989, 11 percent in 1990, and 9 percent in 1991 (Rickman W, Personal communication, 1991).

*Statistical analyses.* Statistical analyses were performed using multiple regression [23], with scores on the Western blot bands, age at seroconversion, and estimated duration of infection as the independent variables and the CD4+ lymphocyte count at initial evaluation as the dependent variable. Zero-order correlations were determined to assess the univariate associations of scores on each band with CD4+ counts and to examine correlations among independent variables. The covariates age and duration of infection were entered into the regression equation first, followed by each band in order of its standardized regression coefficient. Analyses were performed for the entire study period, separately for January 1988 to July 1989 and August 1989 to December 1991, and for individual years. The distribution of CD4+ counts was examined for the presence of extreme values, and the effect of these was assessed by repeating the analyses excluding outliers. All calculations were performed using SPSS-X programs.

## Results

The study included 877 Navy and Marine Corps seroconverters. There were 858 men and 19 women. The mean age was 26.7 (SD,  $\pm 5.7$ ) years. Ninety-four percent had CD4+ lymphocyte counts performed within 90 days of the first positive Western blot. The mean interval between estimated date of seroconversion and the initial CD4+ count was 10.0 (SD,  $\pm 4.3$ ) months. There were 321 seroconverters in 1988, 179 in 1989, 213 in 1990, and 164 in 1991.

The gp120 (envelope) band was strongly reactive in two-thirds of the seroconverters, and the gp160 (envelope precursor) band was strongly reactive in 89 percent (Table 1). By contrast, the p17 core and p55 core precursor bands were strongly reactive in only a quarter.

Table 1. Mean CD4+ lymphocyte counts ( $\pm$  SE) and frequency distribution (and percent) by Western blot band and reactivity score, active-duty U.S. Navy and Marine Corps seroconverters, 1988-1991 (N = 877)

Band	Description		Reactivity score*			
			0	1+	2+	3+
<u>Core and core precursor</u>						
p17	Core	Mean ( $\pm$ SE)	416 ( $\pm$ 41)	565 ( $\pm$ 16)	602 ( $\pm$ 14)	597 ( $\pm$ 15)
		No. (%)	29 (3.3)	235 (26.8)	389 (44.4)	224 (25.5)
p24	Core	Mean ( $\pm$ SE)	425 ( $\pm$ 72)	457 ( $\pm$ 41)	554 ( $\pm$ 17)	605 ( $\pm$ 10)
		No. (%)	8 (0.9)	43 (4.9)	195 (22.2)	631 (71.9)
p55	Core precursor	Mean ( $\pm$ SE)	518 ( $\pm$ 20)	558 ( $\pm$ 14)	621 ( $\pm$ 18)	622 ( $\pm$ 18)
		No. (%)	141 (16.1)	284 (32.4)	232 (26.4)	220 (25.1)
<u>Polymerase components</u>						
p31	Endonuclease	Mean ( $\pm$ SE)	570 ( $\pm$ 57)	596 ( $\pm$ 21)	620 ( $\pm$ 16)	558 ( $\pm$ 12)
		No. (%)	23 (2.6)	166 (18.9)	269 (30.7)	419 (47.8)
p53	Reverse transcriptase	Mean ( $\pm$ SE)	435 ( $\pm$ 83)	551 ( $\pm$ 26)	601 ( $\pm$ 18)	587 ( $\pm$ 10)
		No. (%)	9 (1.0)	113 (12.9)	232 (26.5)	523 (59.6)
p64	Reverse transcriptase	Mean ( $\pm$ SE)	417 ( $\pm$ 87)	530 ( $\pm$ 47)	627 ( $\pm$ 21)	580 ( $\pm$ 9)
		No. (%)	5 (0.6)	42 (4.8)	151 (17.2)	679 (77.4)

Table 1—*Continued*. Mean CD4+ lymphocyte counts ( $\pm$  SE) and frequency distribution (and percent) by Western blot band and reactivity score, active-duty U.S. Navy and Marine Corps seroconverters, 1988-1991 (N = 877)

Band	Description		Reactivity score*			
			0	1+	2+	3+
<u>Envelope components</u>						
gp41	Transmembrane	Mean ( $\pm$ SE)	702 ( $\pm$ 95)	659 ( $\pm$ 20)	582 ( $\pm$ 13)	549 ( $\pm$ 13)
		No. (%)	12 (1.4)	161 (18.4)	353 (40.3)	351 (40.0)
gp120	Envelope	Mean ( $\pm$ SE)	--	567 ( $\pm$ 31)	612 ( $\pm$ 18)	575 ( $\pm$ 10)
		No. (%)	0 (0.0)	55 (6.3)	227 (25.9)	595 (67.8)
gp160	Envelope precursor	Mean ( $\pm$ SE)	--	580 ( $\pm$ 89)	654 ( $\pm$ 27)	577 ( $\pm$ 9)
		No. (%)	0 (0.0)	8 (0.9)	90 (10.3)	779 (88.8)
* Score: 0, absent; 1+, weakly reactive; 2+, moderately reactive; 3+, strongly reactive.						

Strong antibody responses in the p24 (core) ( $p < 0.05$ ), the p53 (reverse transcriptase) ( $p < 0.005$ ), and p55 (core precursor) ( $p < 0.0001$ ) bands were associated with higher initial mean CD4+ counts by multiple regression analysis controlling for age and estimated duration of HIV infection (Table 2). Strong antibody responses to gp41 (transmembrane envelope protein) by contrast, were significantly ( $p < 0.0001$ ) associated with lower CD4+ counts in the regression model (Table 2). A negative, but not statistically significant ( $p = 0.09$ ), association was observed in the regression analysis for the gp160 envelope precursor antigen, with each unit increase in reactivity associated with 51 fewer CD4+ cells (Table 2). Reactivity in the p17, p31, p64, and gp120 bands was not significantly associated with CD4+ lymphocyte counts in the regression model (Table 2). The association of age and duration of infection with the CD4+ count was also not statistically significant.

Regression analyses were also performed separately for the two time periods when testing was done in different laboratories, and for individual years, with similar results (not shown). The direction and strength of the associations were similar in the two time periods. The regression analyses were repeated excluding the single highest and lowest values of the dependent variable, which were the only apparent outliers, and the results were essentially identical. The regression analyses were also repeated grouping individuals with reactivity scores of 0 or 1 on the Western blot, with nearly identical results (not shown).

Table 2. Multiple regression for Western blot bands and initial CD4+ lymphocyte counts, adjusting for age and estimated duration of HIV infection, active-duty U.S. Navy and Marine Corps seroconverters, 1988-1991 (N = 877)

<u>Antibody</u>	<u>Description</u>	<u>Regression coefficient (cells/mm<sup>3</sup>)</u>	<u>Standardized regression coefficient</u>	<u>p-value</u>
<i>Positively associated</i>				
p24	Core	+33.43	+0.08	< 0.05
p53	Reverse transcriptase	+47.70	+0.14	< 0.005
p55	Core precursor	+46.30	+0.19	< 0.0001
<i>Negatively associated</i>				
gp41	Transmembrane	-76.17	-0.23	< 0.0001
<i>Not significantly associated</i>				
p17	Core	+13.89	+0.04	0.28
p31	Endonuclease component	-23.34	-0.08	0.10
p64	Reverse transcriptase	-7.93	-0.02	0.70
gp120	Envelope	+0.66	+0.002	0.97
gp160	Envelope precursor	-51.14	-0.07	0.09
<i>Covariates</i>				
Age	Age in years	-1.19	-0.03	0.42
Duration	Months between seroconversion and Western blot	-0.19	-0.003	0.92
Intercept		698 cells/mm <sup>3</sup>		

Zero-order correlations (Table 3) were highest among four pairs of bands: p53 and p64 ( $r = +0.72$ ), gp120 and gp160 ( $r = +0.64$ ), p31 and gp41 ( $r = +0.62$ ), and p31 and p53 ( $r = +0.55$ ). No other zero-order correlations were stronger than  $r = 0.50$ . Age and estimated duration of infection were not highly correlated with any single band or with each other. The highest correlation involving the covariates was that between duration and gp41 score ( $r = +0.20$ ). No high negative univariate correlations were present among any of the variables.

Table 3. Zero-order correlations between CD4+ lymphocyte counts, Western blot band reactivity scores\*, age, and estimated duration of HIV infection, active-duty U.S. Navy and Marine Corps seroconverters, 1988-1991 (N = 877)

	<u>CD4+</u>	<u>p17</u>	<u>p24</u>	<u>p31</u>	<u>gp41</u>	<u>p53</u>	<u>p55</u>	<u>p64</u>	<u>gp120</u>	<u>gp160</u>	<u>Age</u>	<u>Duration</u>
<u>CD4+</u>	1.00	0.10	0.15	-0.07	-0.16	0.05	0.15	0.01	-0.03	-0.08	-0.06	-0.04
<u>p17</u>		1.00	0.45	0.39	0.34	0.34	0.50	0.26	0.28	0.19	-0.04	0.06
<u>p24</u>			1.00	0.24	0.15	0.25	0.43	0.23	0.22	0.15	-0.11	0.01
<u>p31</u>				1.00	0.62	0.55	0.39	0.50	0.42	0.31	0.06	0.16
<u>gp41</u>					1.00	0.44	0.34	0.39	0.34	0.30	0.05	0.20
<u>p53</u>						1.00	0.23	0.72	0.30	0.24	-0.02	0.07
<u>p55</u>							1.00	0.22	0.29	0.20	0.02	0.13
<u>p64</u>								1.00	0.25	0.27	0.01	0.07
<u>gp120</u>									1.00	0.64	0.02	0.12
<u>gp160</u>										1.00	0.06	0.07
<u>Age</u>											1.00	0.15

\* Score: 0, absent; 1+, weakly reactive; 2+, moderately reactive; 3+, strongly reactive.

## Discussion

An association between the presence and intensity of specific Western blot antibody bands and initial CD4+ lymphocyte counts was observed in active-duty U.S. Navy and Marine Corps personnel following HIV seroconversion. Seroconverters with strong antibody responses to the p55 core precursor and the p24 core protein had higher initial CD4+ counts; conversely, those with strong antibody responses to the gp41 transmembrane protein had lower counts.

The clinical significance of the humoral immune response to HIV is not yet clear. Several possibilities exist. One explanation is that p24 and p55 antibodies may be mediators of an effective immune response to HIV while gp41 antibodies may be detrimental, accelerating early loss of CD4+ lymphocytes. Alternatively, humoral responses may be markers of progression of HIV infection without a causal relationship.

The relationship between p24 antibody reactivity and clinical status has been described previously [2,8-10,24-26]. Cross-sectional studies have reported higher p24 antibody titers in early infection, and prospective studies decreasing titers (and occasional loss of the p24 band) in patients progressing to AIDS [8]. To our knowledge, a specific association between antibody reactivity to the p55 core precursor and initial CD4+ lymphocyte counts or clinical status has not been previously reported. Such an association is reasonable, as both the p24 and p55 antigens are products of the same viral gene [12], and antibodies to p24 have been previously linked with favorable prognosis of HIV infection [24-26], and higher CD4+ lymphocyte counts [27].

This study observed that strong reactivity in the gp41 band was associated with lower initial CD4+ counts. Previous studies have indicated that the gp41 band persisted during advanced stages of AIDS [2,8,10]. An autoimmune component of the disease, stimulated by the presence of gp41 or a similar epitope expressed on CD4+ lymphocytes, might explain this observation. Such an epitope might identify CD4+ lymphocytes or their precursors for cell-mediated cytotoxicity in the circulation, thymus, or elsewhere, reducing the CD4+ cell count. This could result either from infection of the lymphocyte with HIV or from the presence of an antigen on the cell membrane similar to the gp41 glycoprotein.

Studies of the antibody response to HIV may be complicated by the formation of antigen-antibody complexes. This is particularly true in the later stages of HIV infection with antigen excess, which can result in artificially low free antibody levels [28]. Additionally, specific antibody responses to a variety of antigens may be affected by the immunodeficiency itself [10], and loss of antibodies to HIV might reflect the progression of this immunodeficiency. This study evaluated the CD4+ lymphocyte count and antibody reactivity in specific Western blot bands in recent seroconverters relatively early in their HIV infection, suggesting that neither of these possibilities was likely to have accounted for the results.

Core antigens such as p24 and p55 have been reported to vary less than envelope antigens [29], and these results suggest that strong antibody responses to these antigens may have implications for prevention of disease progression [30]. More work is needed to define the role of the humoral immune system in the pathogenesis of progressive HIV infection.

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13. ABSTRACT (Maximum 200 words) The Western blot is the most widely used confirmatory test for diagnosis of human immunodeficiency virus (HIV) seropositivity. Specific bands in the Western blot indicate antibody responses to various portions of HIV or its precursors, and each is assigned a score from 0 to 3+. While the precise role of humoral antibody responses has not been fully established, specific antibody responses might influence the course of HIV infection. This study investigates the association between antibody reactivity to nine principal Western blot bands and initial CD4+ counts among 877 Navy and Marine Corps personnel during 1988-1991. Multiple regression was used evaluate the strength and significance of the associations and to adjust for age and estimated duration of infection. Strong antibody responses to the p24 core (p < 0.05), p53 reverse transcriptase (p < 0.005), and p55 core precursor (p < 0.0001) antigens were associated with higher initial CD4+ counts, with 33-48 additional cells per mm3 associated with each unit increase in the Western blot score, according to a multiple regression analysis which controlled for age and duration of infection (maximum 24 months). By contrast, antibodies to the gp41 transmembrane antigen (p < 0.0001) were associated with lower initial CD4+ counts. Each unit increase in the gp41 band was associated with 76 fewer CD4+ cells per mm3. A negative association was also observed for the gp160 envelope precursor antigen, with each unit increase in reactivity associated with 51 fewer CD4+ cells, although this association was not statistically significant (p = 0.09). Antibodies to the p17 core precursor, the p31 endonuclease, the p64 reverse transcriptase, and the gp120 envelope, were not statistically significantly associated with the CD4+ count in the multiple regression analyses. The positive associations found for p24, p53, and p55 antibodies and CD4+ counts, and the negative association with gp41 antibodies, may have implications in development of a therapeutic vaccine for HIV seropositive patients.				
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